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(71) Applicant *(for all designated States except US)*: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SEED, Brian [US/US]; Apartment 5J, Nine Hawthorne Place, Boston, MA 02114 (US). TING, Adrian [MY/US]; 1675 York Avenue #6G, New York, NY 10128 (US).

(74) Agent: ELBING, Karen, L.; Clark & Elbing, LLP, 176 Federal Street, Boston, MA 02110 (US).

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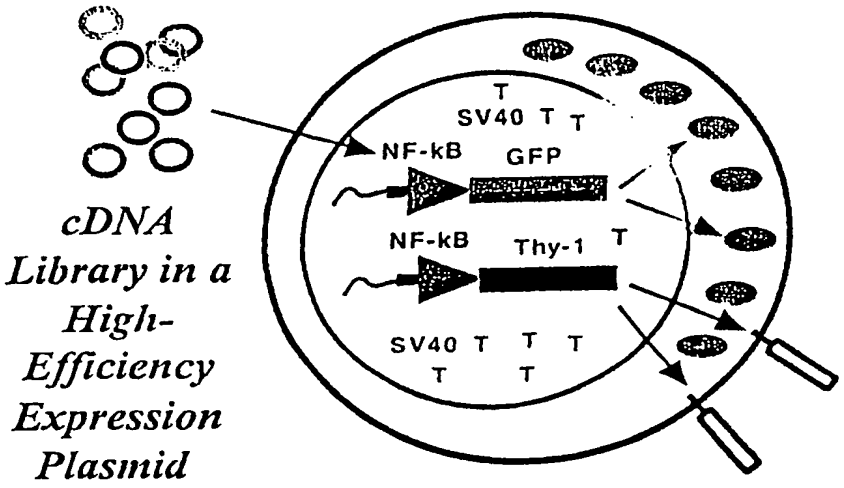

(57) Abstract

The invention features a method of identifying a polypeptide which increases gene expression from a promoter, including (a) contacting a library of polypeptides with a cell that expresses a recombinant anti–cell death gene and that contains a reporter gene operably linked to a promoter (as shown in the figure), whereby expression of the reporter gene is increased if the library includes a polypeptide which increases gene expression from the promoter; (b) determining whether the reporter gene expression is increased in the cell as a result of contact with the polypeptide library; and (c) if reporter gene expression is increased, identifying a polypeptide of the library which increases reporter gene expression.

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METHOD FOR CLONING SIGNAL TRANSDUCTION INTERMEDIATES

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Background of the Invention

The invention relates to methods of identifying polypeptides and compounds which regulate gene expression.

Pharmaceuticals have historically been developed by testing libraries of up to several thousand compounds in laboratory animals, usually one compound at a time. The slow pace of the process, and its unsuitability for screening large numbers of diverse compounds, led to the development of approaches based on assays that can be completed quickly and *ex vivo*. With these approaches, the pharmaceutical drug discovery process has evolved into a catenation of several, sometimes partially concurrent, phases.

In the first phase, target identification and validation, a candidate target for a potential drug is identified by various means. These means may include hypotheses formed from the study of the pathophysiology of disease in humans or experimental animals, analysis of candidate signal transduction pathways *in vitro*, natural experiments such as genetic disorders of humans or other animals, results from targeted or random gene disruptions in model organisms, or disclosures by competitors. The projected consequences of a hypothetical drug interacting with its intended target to stimulate, block or

modulate the target activity is then tested. If the outcome of testing suggests that a drug affecting the target would have the desired physiological consequences in an intact organism, the target is said to be validated. Neither target identification nor validation are formally required, and, indeed, the historical process had neither of these phases.

Present models for pharmaceutical industry productivity emphasize the importance of efficiently identifying large numbers of validated targets and of developing rapid screens for their activity. The impetus for this model is the widespread belief that targets cannot be predicted effectively, so that a large number of targets must be evaluated to develop a small number of drugs. Hence, there remains an important need across the industry to accelerate the pace of target discovery and validation.

Once the target has been identified and validated, an assay must be created to allow large libraries of synthesized or natural compounds to be tested for their ability to interact with the target. When a compound is identified which acts on the target specifically, it is usually said to be a hit. The definition of a hit generally encompasses compounds that pass various secondary tests to assure that their activity is specific to the target of interest. From the various hits that are generated, one or more structures are chosen to represent the starting point for a program of systematic modification of the chemical structure. These structures are called lead compounds, and they are frequently selected from hits on the basis of their compatibility with directed synthesis programs, expected toxicity, or expected absorption, distribution, metabolism or excretion characteristics. Again, the process encompasses a wide range of practices and although they are usually distinguished, hits and lead compounds are sometimes referred to in ways that make them appear equivalent.

Summary of the Invention

In general, the invention provides a novel, rapid method for identifying members of selected signal transduction pathways which are targets for drug design.

In a first aspect, the invention features a method for identifying a polypeptide which increases gene expression from a promoter, including (a) contacting a library of polypeptides with a cell that expresses a recombinant anti-cell death gene and that contains a reporter gene operably linked to a promoter, whereby expression of the reporter gene is increased if the library includes a polypeptide which increases gene expression from the promoter; (b) determining whether the reporter gene expression is increased in the cell as a result of contact with the polypeptide library; and (c) if reporter gene expression is increased, identifying a polypeptide of the library which increases reporter gene expression.

In a second aspect, the invention features a method for identifying a polypeptide which decreases gene expression from a promoter, including (a) contacting a library of polypeptides with a cell that expresses a recombinant anti-cell death gene and that contains a reporter gene operably linked to the promoter, whereby expression of the reporter gene is decreased if the library includes a polypeptide which decreases gene expression from the promoter;

(b) determining whether reporter gene expression is decreased in the cell as a result of contact with the polypeptide library; and

(c) if reporter gene expression is decreased, identifying a polypeptide which decreases reporter gene expression.

In a third aspect, the invention features a method for identifying a polypeptide which modulates activation of a transcription factor activation domain, including (a) contacting a library of polypeptides with a cell that

expresses a recombinant anti-cell death gene and a chimeric transcription factor consisting of a yeast or bacterial DNA binding domain fused to a mammalian transcription factor activation domain, and that contains a reporter gene operably linked to a promoter consisting of a basal promoter and binding sites for the DNA binding domain, whereby expression of the reporter gene is altered if the library comprises a polypeptide which modulates activation of the transcription factor activation domain; (b) determining whether reporter gene expression is altered in the cell as a result of contact with the polypeptide library; and (c) if reporter gene expression is altered, identifying a polypeptide which modulates reporter gene expression.

In a fourth aspect, the invention features a method for identifying a compound which modulates gene expression from a promoter, including (a) contacting a library of compounds with a cell that expresses a recombinant anti-cell death gene and that contains a reporter gene operably linked to the promoter, whereby expression of the reporter gene is altered if the library includes a compound which modulates gene expression from the promoter;

(b) determining whether reporter gene expression in the cell is altered as a result of contact with the compound library; and

(c) if reporter gene expression is altered, identifying a compound from the library which modulates reporter gene expression.

In a fifth aspect, the invention features a method for identifying a compound which decreases gene expression, including (a) contacting a library of compounds with a cell expressing (i) a recombinant anti-cell death gene; (ii) a second gene encoding a polypeptide; and (iii) a reporter gene that would have decreased expression if the function of the polypeptide was blocked; (b) determining whether expression of the reporter gene is decreased as a result of contact with the compound library; and (c) if expression of the reporter gene is

decreased, identifying a compound from the library which decreases reporter gene expression.

In preferred embodiments of the first, second, and third aspects, a library of DNA molecules encoding the library of polypeptides are expressed in a cell. The cell can be the same cell that expresses a recombinant anti-cell death gene and that contains a reporter gene operably linked to a promoter (in which case the polypeptide is produced by the same cell that expresses a recombinant anti-cell death gene and that contains a reporter gene operably linked to a promoter), or the polypeptide can be produced by a cell other than the cell that expresses a recombinant anti-cell death gene and that includes a reporter gene operably linked to a promoter; preferably the DNA molecules are expressed from a high-efficiency expression system.

In other preferred embodiments of the first, second, and third aspect, the library of DNA molecules is introduced to the cell by transfection, and the mean number of DNA molecules introduced by transfection to the cell is at least 25. Preferably, the mean number of DNA molecules introduced by transfection into the cell is at least 100 or even 500.

In still other preferred embodiments of the first, second, and third aspect, the polypeptide is selected from the group consisting of an extracellular ligand, a cell surface receptor, and a signal transduction intermediate, and the DNA molecules are expressed from a high-efficiency expression system.

In preferred embodiments of the first, second, third, fourth, and fifth aspects, step (c) includes (i) dividing the library into two or more libraries with less complexity; and (ii) repeating steps (a) and (b) until a polypeptide which activates reporter gene expression is identified; the promoter can derived from a mammal and the library of polypeptides can include polypeptides derived from a bacterium or a virus.

In other preferred embodiments of the first, second, third, fourth, and fifth aspects, the promoter is a heterologous promoter, the reporter gene is GFP, the anti-cell death gene is selected from the group consisting of bcl family members, IAP family members, and crmA, and the cell is selected from the group consisting of CHO, CD-1, Cos, 293, HeLa, BHK, or L cells.

In another aspect, the invention features a method for determining whether a compound modulates NF-kB biological activity, the method including the steps of: (a) providing a cell expressing a BCMA polypeptide; (b) contacting the cell with a candidate compound; and (c) measuring the level of expression of the BCMA polypeptide in the cell, wherein a change in the level of expression of the BCMA polypeptide in the cell, relative to a cell not contacted with the candidate compound, identifies the candidate compound as a compound that modulates NF-kB activity.

In still another aspect, the invention features a method for determining whether a compound modulates BCMA biological activity, the method including the steps of: (a) providing a BCMA polypeptide; (b) contacting the polypeptide with a candidate compound; and c) measuring the level of biological activity of the BCMA polypeptide, wherein a change in the level of biological activity of the BCMA polypeptide, relative to a polypeptide not contacted with the candidate compound, identifies the candidate compound as a compound that modulates BCMA activity. The BCMA polypeptide can be in a cell or in a cell-free system. A preferred BCMA biological activity is the modulation of NF-kB biological activity (e.g., the modulation of transcription by NF-kB) or NF-kB expression. Preferably, the BCMA polypeptide includes a polypeptide sequence having substantial identity to amino acids 98 to 164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2).

In yet another aspect, the invention features a method for determining whether a compound modulates NF-kB activity, the method including the steps of: (a) providing a BCMA polypeptide; (b) contacting the polypeptide with a candidate compound; and (c) detecting the binding of the candidate compound to the polypeptide, wherein a candidate compound that binds to the polypeptide is a compound that modulates NF-kB biological activity. The BCMA polypeptide can be in a cell or in a cell-free system. Preferably, the BCMA polypeptide includes a polypeptide sequence having substantial identity to amino acids 98 to 164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2).

In another aspect, the invention features a substantially purified polypeptide consisting of a BCMA polypeptide molecule lacking a BCMA extracellular domain. Preferably, the BCMA polypeptide includes a polypeptide sequence having substantial identity to amino acids 98 to 164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2). Other preferred polypeptides are BCMA polypeptides that modulate NF-kB activity.

In yet another aspect, the invention features an NF-kB modulator including a BCMA polypeptide covalently linked to a heterologous compound. Preferably, the BCMA polypeptide includes a polypeptide sequence having substantial identity to amino acids 98 to 164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2). Preferably, the modulator modulates NF-kB biological activity or expression.

The invention also features methods for activating NF-kB activity in a cell by contacting the cell with a recombinant BCMA polypeptide having NF-kB activating activity or by contacting the cell with a recombinant nucleic acid molecule encoding a BCMA polypeptide having NF-kB activating activity.

In related aspects, the invention features the use of BCMA polypeptides and nucleic acids for preparing pharmaceutical compositions for treating cancer or apoptosis.

By a "reporter gene" is meant a DNA or RNA sequence which encodes a reporter protein that is capable of being readily detected either inside or outside a cell.

The reporter gene is operably linked to a promoter which shows low spontaneous activation, so that the activity of the reporter protein in the presence of the activating polypeptide be at least two standard deviations greater than the activity in its absence. Various methods of increasing the sensitivity of reporter genes are known in the art, including: deletion from natural genes of inhibitory sequences, which may be found both within and external to the transcribed portion and which may affect mRNA formation, stability, or translational efficacy; addition of efficiently utilized introns to increase the rate of formation of mature mRNA; multimerization of upstream activation regions or binding sites for known transcriptional activators; increasing the copy number of the reporter gene; and protection of the activity of the reporter gene from adventitious stimulatory or inhibitory activity in flanking DNA by inserting the reporter gene between matrix attachment regions or chromatin insulator sequences.

Many different types of reporter proteins are known in the art. They frequently comprise proteins not normally found, or present in minor amounts, in some cells; they include enzymes that detoxify antimicrobial agents, such as aminoglycoside or aminocyclitol phosphotransferases or acetyltransferases, beta-lactamases or chloramphenicol acetyltransferase; enzymes of diverse origin that catalyze chromogenic, fluorogenic, or chemiluminescent reactions in the presence of exogenous substrates, such as beta-galactosidase, beta-

glucuronidase, alkaline phosphatase, catechol 2,3-dioxygenase, or various peroxidases; enzymes that catalyze photoreactions, such as bacterial or firefly luciferases; enzymes, like glycosyl transferases, that generate nonproteinaceous structures easily detected by antibodies, lectins, or cognate binding proteins; proteins easily monitored upon cell surface expression or secretion such as surface or secreted antigens for which corresponding antibodies or recognition proteins are known; and proteins which catalyze the synthesis of, or stoichiometrically embody, fluorescent structures, without exogenous substrates, such as the jellyfish fluorescent proteins (e.g., GFP).

By "operably linked" is meant that the gene for the reporter protein is positioned adjacent to a promoter which directs transcription of the gene and, ultimately, facilitates expression of the reporter protein.

By "promoter" is meant any minimal nucleic acid sequence sufficient to direct transcription of the reporter gene. The promoter is one which is activated by binding to a polypeptide. Examples of promoters useful in the invention are promoters which are normally linked to genes which are expressed when a cell is in a pathologic or disease state (e.g., cancer, inflammation, or due to bacterial or viral infection), and the protein products of which are directly or indirectly responsible for this state. Suitable promoters include, but are not limited to, the NF-kB promoter, the interleukin-2 promoter, and the HIV-1 long terminal repeat promoter.

A library of DNA molecules refers to a set of DNA molecules, each in a DNA expression vector. Preferably, the DNA expression vector displays high efficiency such that the level of expression is high. While in most cases, the library includes DNA molecules encoding tens, hundreds, or even thousands of different polypeptides, DNA molecules in a library can also encode only one polypeptide (for example, during the final steps of a sib

selection). A library with hundreds of different DNA molecules is considered to have greater "complexity" than a library with five different DNA molecules. Like DNA libraries, compound libraries can have different degrees of complexity. One feature of the invention is a method which allows for following an activity of interest through the sequential screening of libraries with less and less complexity.

By "candidate compound" is meant a chemical, be it naturally-occurring or artificial, that is surveyed for its ability to modulate BCMA or NF-kB biological activity. Candidate compounds may include, for example, peptides, polypeptides, antibodies (and fragments thereof), synthesized organic molecules, naturally occurring organic molecules, nucleic acid molecules, and components or derivatives thereof. The candidate compounds may be screened using any of the methods described herein using an animal, a cell, a lysate or extract derived from a cell, or a molecule derived from a cell. The measuring may be, for example, for the purpose of detecting altered gene expression, altered RNA stability, altered protein stability, altered protein levels, altered protein phosphorylation, or altered protein biological activity. The means for measuring may include, for example, antibody labeling, immunoprecipitation, phosphorylation assays, and methods known to those skilled in the art for detecting nucleic acids.

By "modulating" is meant changing, either by decrease or increase.

By "BCMA polypeptide" is meant a polypeptide that has substantial identity to human or mouse BCMA as shown in Figs. 7A and 7B, respectively, over a region of twenty consecutive amino acids and has a BCMA biological activity.

By "substantial identity" is meant that two polypeptide sequences, when optimally aligned, such as by the GAP or BESTFIT programs using

default gap weights, share at least 80% sequence identity, more preferably at least 90% sequence identity, and most preferably at least 95% or even 99% sequence identity or more.

By "heterologous compound" is meant a polypeptide, chemical, or detectable label (e.g., biotin) that is not naturally associated, in this case, with the polypeptide.

By "BCMA biological activity" is meant BCMA-mediated modulation of NF-kB biological activity, or expression or the binding of an antibody that specifically binds a BCMA polypeptide.

By "NF-kB biological activity" is meant any function performed by activated NF-kB.

The invention features a method of screening DNA and compound libraries for their ability to modulate reporter gene expression in a cell which is expressing a recombinant anti-cell death gene. The expression of this gene allows the cell to survive in conditions which would otherwise lead to its death and, as a result, failure of the screening procedure.

This method is suitable for identifying polypeptides that modulate transcription from a selected promoter. These polypeptides are, in turn, targets for drugs. The method is also suitable for identifying compounds which either mimic or block function of a polypeptide which itself modulates transcription from a selected promoter.

Hence, the invention features a new, efficient multistep method for identifying lead compounds which modulate expression from a promoter for development of pharmaceutical compounds.

The invention also features a new NF-kB activator, BCMA. As NF-kB is involved in numerous cellular processes and disease states, BCMA is useful for the treatment of disease. BCMA is also useful for the identification

of compounds that modulate its expression or biological activity. These compounds may be developed as drugs, or used as lead compounds for the purpose of identifying drugs.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

Brief Description of the Drawings

Fig. 1 is a schematic illustration of a reporter cell system for rapid identification of cDNAs encoding polypeptides of interest. A cDNA library is transfected into a cell which includes a reporter gene (e.g., GFP or Thy-1) operably linked to a promoter (in this case the NF-kB promoter). If the polypeptide expressed by one of the plasmids in the reporter cell is capable of inducing expression from the NF-kB promoter, the reporter gene is expressed, and the detectable marker protein is produced.

Fig. 2 is a schematic illustration of a cDNA library being divided into smaller libraries. Each of the libraries is then transfected into reporter cells. Libraries which induce reporter gene expression are then further divided until the cDNA(s) encoding the polypeptide which induces reporter gene expression are isolated.

Fig. 3 is a schematic illustration of three stages of enrichment for an intracellular signal transduction intermediate. The ratios represent the occurrence of the cDNA encoding the reporter gene-inducing polypeptide compared to the total cDNAs. Note that, during transfection, each cell receives more than one plasmid.

Fig. 4 is a schematic illustration showing how secreted factor can be distinguished from intracellular signal transduction intermediates such as receptors or kinases. Soluble ligands diffuse through the medium to engage

receptors on untransfected cells, which can lead to reporter gene expression in most, if not all, cells. Intracellular effectors are restricted to the transfected cells.

Fig. 5 is a schematic illustration of arrayed clones. N^2 elements, pooled into N columns and N rows, can be assayed with 2N transfections. In the example provided, 4 rows and 4 columns were positive. 4^2 or 16 transfections are then required to identify the intersections unambiguously.

Fig. 6 is a schematic illustration of an indexed library protocol. The method is similar to the one described in Fig. 1 to Fig. 5, except that individual plasmids are grown as individual cultures. Libraries of plasmids are then prepared and assayed. This method facilitates the rapid recovery of individual plasmids, and prevents the loss of positives when a library is divided into libraries of lower complexity.

Fig. 7A is a schematic illustration showing human BCNA polypeptide sequence (GenBank accession number Q02223). The putative transmembrane domain is indicated in bold.

Fig. 7B is a schematic illustration showing mouse BCNA polypeptide sequence (GenBank accession number AAC23799).

Fig. 8 is a schematic illustration showing that the intracellular domain of human BCMA was capable of activating NF-kB. A series of fusion proteins were constructed using the CD5L leader sequence, IgG Fc, a CD7 transmembrane domain, and amino acids from human BCMA (SEQ ID NO: 1) as follows: Ig7bcma--amino acids 78-184; ΔC20--amino acids 78-164; ΔC40--amino acids 78-144; ΔC60--amino acids 78-124; ΔC80--amino acids 78-104; ΔN20--amino acids 98-184; ΔN40--amino acids 118-184; ΔN60--amino acids 138-184; ΔN80--amino acids 158-184.

Detailed Description

The present invention provides a method for the rapid identification of molecules involved in regulation of specific signal transduction pathways. It can be used both as a target identification tool and as a rapid assay for drug effectiveness, and as such can significantly reduce the amount of time needed to go from target to hit.

The method features an expression cloning approach that identifies polypeptides that have the ability to activate reporter genes (Fig. 1). Such polypeptides are called here activating polypeptides. Expression cloning is a technique which identifies polypeptides solely on the basis of their ability to generate an observable activity of interest. In order for that activity to be identified, the polypeptide must generally be expressed, either *in vivo* or *in vitro*, and a suitably sensitive assay must be available to detect the activity after the polypeptide has been expressed. In the present method, it is preferred that the activity that is detected is dependent on the output of a reporter gene (Fig. 1).

Although the use of expression cloning in conjunction with reporter genes is not new, existing methods of application have not resulted in highly efficient systems for the identification of large numbers of new molecules. The present approach is a high throughput system for the identification of cDNA clones encoding polypeptides that induce reporter gene activity. Some features of the high throughput system of the present invention are: (1) a very high efficiency cDNA expression plasmid; (2) an easily detected reporter molecule; (3) features to prevent the death of the reporter cell due to toxicity of expressed genes; and (4) a method for the introduction of large numbers of plasmids into multiple cells.

Expression cloning can be carried out by either of two general

paradigms, positive selection, or sib selection, also known as pool division. By positive selection is meant a method for the enrichment of cells, viruses or genetically linked assemblies of proteins and the nucleic acids that encode them by physically separating the cells or genetically linked assemblies from the much larger collection of cells or assemblies that encode molecules of little interest. Because the nucleic acids that encode the polypeptides of interest are physically linked to the cells, viruses, or other assemblies that they comprise, the nucleic acid is physically purified by positive selection and the process can be repeated until a single nucleic acid is found that encodes the activity of interest. It is desirable in positive selection schemes to develop methods for association of a single nucleic acid with a single protein assembly or cell. Thus in positive selection it is desirable to identify and use methods for the introduction of nucleic acids into cells that result in a single nucleic acid species per cell.

The method of the invention uses sib selection, a process of screening in which pools of molecules that are derived from cognate libraries of nucleic acids are assayed for activity, and positive libraries are detected by some signal (e.g., expression of a reporter gene). The nucleic acids that make up the library are then separated into libraries with less complexity, which are then reassayed and redivided until a single nucleic acid is found that encodes the polypeptide with the desired activity (Figs. 2, 3, and 4) Because sib selection depends on the detection of the activity of aggregates of nucleic acids, it is often advantageous to use methods for the transfection or introduction of nucleic acids into cells that result in a large number of nucleic acid species per cell. Sib selection schemes, like positive selection methods, can also be carried out entirely *in vitro*.

Features of the Invention

Transfection Method

For sib selection to be an efficient method of cloning genes, according to the invention, it is important that a large number of DNA molecule are introduced into each cell. Many methods of introducing DNA molecules are known in the art, including microinjection, complexation with positively charged synthetic polycations such as DEAE dextran, polybrene, polylysine, or polyarginine, complexation with histones and other basic proteins, complexation with cationic lipids or related amphipathic molecules, condensation with polyethylene glycol or polyhydroxybutyrate, coprecipitation with calcium phosphate, electroporation, scrape loading or partial rupture, and fusion with bacterial or microbial spheroplasts. Preferred among these are methods that can be easily carried out in parallel and that result in the co-introduction into the reporter cell of multiple nucleic acids that encode different species. Moreover, the method of transfection will preferably provide, on average, at least 25 DNA molecules per cell. More preferably, the mean number will be at least 100 or even 500 DNA molecules per cell. The methods which achieve the preferred results include calcium phosphate coprecipitation, complexation with polycations or cationic lipids, and condensation with uncharged polymers such as polyethylene glycol.

The libraries of nucleic acid molecules encoding potentially activating polypeptides can also be created in a biologically active assembly, such as a virus or viral transducing particle, which is capable of introducing itself into the reporter cell directly. In such a case a cDNA library is prepared in the viral vector, and libraries of active virus or transducing particles are applied to the reporter cell. In general the reporter cell will have been previously engineered to contain a reporter gene, but the reporter gene may also

be introduced concurrently with the activating nucleic acid.

High Efficiency cDNA Expression Vector

To detect ectopic activation of a signal transduction pathway it is desirable to greatly overexpress proteins that act as signal transduction intermediates.

The invention uses a high efficiency cDNA expression system to produce proteins in the reporter cell. Such a system is provided by the use of a very strong promoter, such as, for example, the elongation factor 1 alpha (EF-1α) promoter; 3' untranslated region (3' UTR) and polyadenylation consensus (poly(A)) sequences from the human growth hormone gene; and the human IgG1 hinge-CH2 intron. Other strong promoter and nonpromoter elements are known in the art (for example, the murine or human cytomegalovirus immediate early gene promoters, globin introns, and 3' UTR/poly(A) sequences).

Cell Death Inhibition

Another important feature of the present system is a method to prevent the death of cells overexpressing proteins. Such a method is important for two reasons: signal transduction intermediates themselves can lead to cell death if they are expressed at high levels, and, in addition, if libraries of nucleic acids are transfected, the presence of even a low frequency of nucleic acids encoding toxic proteins can interfere with detection of the desired signal. The latter effect can be predicted to have greater impact as the size of the library increases.

There are several known methods to prevent the demise of cells undergoing programmed cell death, or apoptosis. Both viral and cellular

antagonists of cell death are known, and among them are species that act upstream in the apoptosis pathway, or at multiple levels. Examples of the former include dominant negative forms of cell death proteins, such as FADD or TRADD, and cellular inhibitors of apoptosis, such as members of the viral or cellular IAP family. Examples of proteins that act at multiple levels, or relatively downstream, include the orthopoxvirus CrmA and baculovirus p35 proteins, members of the cellular Bcl family, and both peptide and nonpeptide inhibitors of caspases, the cysteine proteases which act in a zymogen cascade to generate the death program. For the prevention of apoptosis in expression cloning, combinations of broad spectrum antiapoptotic proteins with different mechanisms of action are preferred, such as CrmA and Bcl-xL.

Examples

Identification of gene products leading to the activation of the transcription factor NF-kB

We established a reporter cell line by stably transfecting the commercially-available human embryonic kidney cell line, 293 EBNA, with two constructs: a reporter construct consisting of NF-kB promoter elements upstream of the green fluorescent protein (GFP); and a eukaryotic expression construct that expresses two anti-cell death molecules, CrmA and Bcl-xL. The former functions as a readout for the presence of signals that activate the NF-kB signal transduction pathway, whereas the latter prevents these cells from undergoing programmed cell death in the event that pro-apoptotic signals are present. The expression in the reporter cell line of NF-kB-activating molecules, including receptors (e.g. tumor necrosis factor-α receptor 1), ligands (e.g. tumor necrosis factor-α) and intracellular signaling proteins (e.g. RIP), results in the robust production of GFP. The level of GFP production, as a

measure of NF-kB activation, can be assayed using a fluorescent microscope or flow cytometry. High level expression of these various NF-kB-activating molecules was achieved by placing their cDNAs under the control of the very strong EF-1α promoter present in the commercially available plasmid PEAK8 (Edge Biosystems). Thus, one can transfect into these cells cDNA libraries and identify individual cDNAs which are capable of NF-kB activation.

A cDNA library, prepared from activated human T cell mRNA and cloned into the PEAK8 vector, was subdivided into smaller libraries of approximately 500 cDNA clones each, and DNA was prepared from each library. The DNA from the libraries were transfected into the reporter cell line by calcium phosphate precipitation following published protocols, e.g., Ausubel et al. et al., 1997, *Current Protocols in Molecular Biology*, Wiley Interscience. Cells were allowed to continue to grow for 48 hours and assayed for GFP production. cDNA libraries that gave a positive GFP signal, as defined by the appearance of at least 0.1% of bright cells by fluorescence microscopy, were screened further by sub-dividing into libraries of 50 cDNAs each (Fig. 2). DNA from each library was prepared, transfected into the reporter cell line and assayed for GFP production. The process was repeated until a single cDNA clone that induced a positive GFP readout was obtained. The screening procedure has resulted in the cloning of DNAs encoding known NF-kB-activating molecules such as surface receptors (DR3, FAS), soluble ligands (interleukin-1, TRAIL, CD40 ligand), intracellular signaling molecules (small molecular weight GTPase rho). Also identified were a previously known molecule having no known function (BCMA) and novel molecules.

The inclusion of two anti-cell death genes in the transfected cells are likely to have aided in the expression cloning of at least some of the above-mentioned DNAs. It has been established that expression of either FAS or DR3

in a cell would induce apoptosis in that cell. The fact that each was retrieved substantiates the improved nature of the expression cloning method of the present invention. Similarly, as the method of the present invention preferably employs high copy number, it is likely that expression of anti-cell death genes allows for cells that otherwise would have died, due to a high number of inserts, to survive.

The method is more rapidly and efficiently applied by using indexed arrays of bacterial cultures, in which each culture is derived from a single colony, and, thus, represents an independently derived cDNA expression plasmid (Fig. 5). By pooling small amounts of the cultures from rows and columns of large arrays and preparing DNA from each of the libraries, it is possible to assess the relative activity of every row and every column, thereby reducing labor dramatically (Fig. 6). For example if there are 90,000 individual/clones arrayed in a 300 by 300 matrix, the 300 rows and 300 columns can be transfected to determine all of the rows and all of the columns that bear positive clones. Hence the array can be indexed in only 600 DNA preparations and transfections. If there is only one positive culture, the job is complete. If there are multiple positive cultures, then in the worst case, each row and each column will have only one positive culture, and if there are x positives, then there are x^2 possible intersections. As long as x is a small number, though, the work involved is quite modest. For example if x were 10, the entire array of 90,000 clones could be screened in only 700 DNA preparations and transfections.

Variations of the experimental approach outlined above are applicable depending on the particular system that is to be examined. The reporter system and cell lines can be adapted to the promoter to be investigated. For instance, the NF-kB promoter in the GFP reporter construct can be replaced

by the interleukin-2 promoter, introduced into a T lymphocyte cell line and screened for molecules that regulate the signal transduction pathway leading to the expression of interleukin-2 in T lymphocytes. Other promoters can be drawn from viral sources, such as the HIV-1 long terminal repeat (LTR) promoter, or other inducible promoters of viral origin. The calcium phosphate transfection procedure can be changed to another method more suitable for the particular reporter cell type. The alternative transfection protocols could be based on electroporation, cationic lipids, DEAE-dextran, spheroblasts fusion or viral-mediated delivery.

A variation to the method described above is to screen for molecules that will turn off expression from a promoter. For instance, the NF-kB reporter in the reporter cell line is activated in response to interleukin-1 stimulation. By including interleukin-1 in the culture medium, one can then search for molecules that will inhibit the interleukin-1 dependent expression from the NF-kB promoter. Depending on the cDNA library that is used, these molecules can be either ones that are naturally negative regulatory or they can be mutant versions that behave in a dominant inhibitory manner. Similarly, screens can be conducted with a combinatorial library to look for small pharmacological molecules that will negatively interfere with the pathway (see below).

The screening methods described above are well-suited for screening for genes from one organism that interact with a pathway in another organism. A prime example is screening the expressed genomes of viruses, bacteria or other pathogens for genes that, when expressed, might interact with the NF-kB pathway, This method, utilized by these pathogens to alter the immune response to their advantage, can identify potential targets for pharmacological interventions.

The reporter cell may also be provided with proteins which increase

the efficiency of the reporter gene. These proteins are usually introduced by transfection of an expression plasmid that encodes them. Proteins which increase the efficiency of the reporter gene may provide essential elements of a signaling pathway which are not otherwise present in the reporter cell, or may consist of artificial proteins that act to integrate, amplify, or selectively respond to signals from the pathway of interest. Of special relevance are artificial transcriptional activators which consist of a DNA binding element that interacts specifically with cognate binding sites in an artificial promoter and a pathway-specific transcriptional activator element that responds to activation of the pathway with a change in transcriptional activity. Such artificial activators are known in the art as fusions between bacterial or yeast DNA binding proteins and mammalian transcription factor activation domains. Such activation domains provides sites for protein binding, ligand-activated conformational change or post-translational modification, that increase the transcription-promoting capabilities of the artificial activator. Examples of artificial activators that are commercially available or known in the art include LexA and Gal4 fusions with c-Jun, Elk1, CREB, c-Fos, ATF2, CHOP, and members of the nuclear hormone superfamily.

In one example, a cell which contains (i) a recombinant anti-cell death gene, (ii) a chimeric transcription factor consisting of the Gal4 DNA binding domain fused to the c-Jun activation domain, and (iii) DNA encoding GFP operably linked to regulatory sequence consisting of a basal promoter and Gal4 binding sites is used to identify polypeptides or compounds which modulate c-Jun activation. If, for example, expression of a polypeptide leads to c-Jun phosphorylation (i.e., activation), then increased GFP expression would result.

BCMA is an activator of NF-kB

Using the methods described herein, we identified BCMA as an NF-kB activator. BCMA was discovered by molecular analysis of a t(4;16) translocation characteristic of a human T cell lymphoma (Laabi et al., EMBO J. 11: 3897-3904, 1992), and its function was not previously known.

Based on the present discovery, BCMA is a target for drug discovery or rational drug design. For example, a compound that modulates BCMA expression or biological activity will also modulate NF-kB biological activity. Accordingly, the invention features methods and reagents for the identification of NF-kB modulating compounds.

BCMA polypeptides or nucleic acid molecules are also useful for the treatment of diseases associated with insufficient or inappropriate NF-kB biological activity or expression. BCMA polypeptides or nucleic acid molecules are administered to a patient using an appropriate delivery vehicle, as known in the art. Generally, the BCMA polypeptide or nucleic acid molecule is delivered in a pharmaceutically acceptable carrier.

Coupled Target Identification and Assay Generation

Directly relevant to the NF-kB signal transduction pathway is the identification of molecules that could potentially play a role in regulating inflammation and oncogenesis. The search for NF-kB activating molecules do not have to be restricted to screening cDNA expression libraries; the same principles can be used to identify compounds which modulate the output of the pathway, either by mimicking the activity of a polypeptide or by blocking its activity when the polypeptide is overexpressed. The latter compounds will act either upon or downstream of the overexpressed protein; once enough activating proteins have been identified, it will be possible to identify at what

step the compounds act by evaluating their action on a panel of transfected cells expressing different activating proteins.

In general, compounds are identified from large libraries of both natural product and synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic

dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known to modulate the test promoter should be employed whenever possible.

When a crude extract is found to modulate reporter gene expression, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having the desired activity. Methods of fractionation and purification of such heterogenous extracts are known in the art. If desired, compounds shown to be useful agents for the modulation of gene expression or biological activity are chemically modified according to methods known in the art.

Primary screens for compounds that modulate BCMA biological activity

Modulating NF-kB expression or biological activity modulates numerous important cellular processes. The finding that NF-kB activity is regulated by BCMA allows us to provide assays for drugs that modulate NF-kB by monitoring BCMA expression or biological activity. Such assays may measure BCMA expression by measuring changes in: (a) levels of BCMA protein; (b) levels of BCMA RNA; (c) levels of BCMA-mediated NF-kB biological activity; or (d) levels of a reporter gene or protein expressed from a NF-kB promoter. These measurements may be made *in vitro* or *in vivo*. These assays allow for the identification of compounds that modulate NF-kB biological activity (e.g., gene transcription). Such identified compounds may have therapeutic value, for example, in the treatment of diseases that result in too little or too much cell death.

Cells overexpressing BCMA can be produced using standard techniques. Compounds that are identified may bind to BCMA and prevent BCMA activation of NF-kB. While screening of compounds can be performed with cultures of primary cells, cell lines be also be used. Cell lines can be modified such that the cells constitutively express a BCMA polypeptide, for example, the BCMA intracellular domain.

Any cell line, such as ones described above, can also be engineered to contain a reporter gene expressed under control of the NF-kB promoter (described above). A preferred reporter gene codes for GFP. Typically, the expression of the gene (e.g., the endogenous NF-kB gene or a recombinant reporter gene expressed under the control of the NF-kB promoter or fragment thereof) is measured by assaying the RNA or protein levels or both of the expressed gene. For example, the polypeptide expressed by the NF-kB gene or by the reporter gene produces a detectable signal under conditions that allow compound-mediated changes to be measured. Quantitatively determining the amount of signal requires comparing the amount of signal produced in the absence of any compound being tested to the amount produced when the cell is contacted with the compound, as is described herein. The comparison permits the identification of the compound as one that causes a change in the detectable signal produced by the expressed gene (e.g., at the RNA or protein level) and thus identifies a compound that is capable of modulating NF-kB expression. In order to prevent the NF-kB cells from dying, a second gene encoding an apoptosis inhibitor can also be expressed in the cells, as described herein.

Secondary screens for compounds that modulate NF-kB activity

After test compounds that appear to modulate NF-kB expression are identified, it may be necessary or desirable to subject these compounds to

further testing. The invention provides such secondary confirmatory assays. For example, a compound that appears to modulate NF-kB activity in early testing may be subject to additional assays to confirm that the compound also modulates NF-kB activity *in vivo*. In the first round of *in vivo* testing, NF-kB activity is initiated in animals by well-known methods and the compound is administered by one of the means described in the "Therapy" section, immediately below. Cells or cellular tissue are isolated within hours to days following the insult, and are subjected to assays to assess the level of NF-kB expression or biological activity. Such assays are well known to those skilled in the art. Examples of such assays include, but are not limited to, ELISAs, Western blot analysis, RT-PCR, RIA, and Northern blot analysis.

Therapy:

NF-kB is an important regulator of inflammatory responses (e.g., rheumatoid arthritis, inflammatory bowel disease, septic shock), apoptosis, oncogenesis, and anti-viral and anti-bacterial responses. Therefore, the discovery of new gene products that regulate NF-kB activity, and thus the disease process, will result in the identification of molecular targets for pharmacological intervention. By increasing or mimicking BCMA biological activity, one could, for example, boost anti-tumor antibody production or increase T cell cytotoxicity against tumor cells in cancer immunotherapy. Conversely, antagonizing BCMA biological activity would be advantageous, for example, in situations in which it is desirable to down-regulate immune cell function. Compounds, identified using any of the methods disclosed herein, may be administered to patients or experimental animals with a pharmaceutically- acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable

formulations or compositions to administer such compositions to patients or experimental animals. Although intravenous administration is preferred, any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (19th ed.) ed. Gennaro AR., 1995, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethyl ene-polyoxypropyl ene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for antagonists or agonists of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

As described herein, we have discovered that BCMA activates NF-kB activity. NF-kB, in turn, activates numerous cellular processes. Hence, any compound that modulates NF-kB expression is a candidate compound for use

in drug development. One possible compound is a polypeptide fragment of BCMA which maintains its ability to bind to another component of the signal transduction pathway but has lost its ability to activate NF-kB. Such a polypeptide will act as an inhibitor of wild-type NF-kB signaling . Another possible compound is a polypeptide fragment of BCMA which exhibits constitutive activation of NF-kB.

Other Embodiments

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departments from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

What is claimed is:

1. A method for identifying a polypeptide which increases gene expression from a promoter, said method comprising the steps:

(a) contacting a library of polypeptides with a cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter, whereby expression of said reporter gene is increased if said library comprises a polypeptide which increases gene expression from said promoter;

(b) determining whether said reporter gene expression is increased in said cell as a result of contact with said polypeptide library; and

(c) if said reporter gene expression is increased, identifying a polypeptide of said library which increases said reporter gene expression.

2. The method of claim 1, wherein step (c) comprises (i) dividing said library into two or more libraries with less complexity; and (ii) repeating steps (a) and (b) until a polypeptide which activates reporter gene expression is identified.

3. A method for identifying a polypeptide which decreases gene expression from a promoter, said method comprising:

(a) contacting a library of polypeptides with a cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter, whereby expression of said reporter gene is decreased if said library comprises a polypeptide which decreases gene expression from said promoter;

(b) determining whether said reporter gene expression is decreased in said cell as a result of contact with said polypeptide library; and

(c) if reporter gene expression is decreased, identifying a polypeptide

which decreases said reporter gene expression.

4. The method of claim 3, wherein step (c) comprises (i) dividing said library into two or more libraries with less complexity; and (ii) repeating steps (a) and (b) until a polypeptide which decreases reporter gene expression is identified.

5. A method for identifying a polypeptide which modulates activation of a transcription factor activation domain, said method comprising:

(a) contacting a library of polypeptides with a cell that expresses a recombinant anti-cell death gene and a chimeric transcription factor consisting of a yeast or bacterial DNA binding domain fused to a mammalian transcription factor activation domain, and that comprises a reporter gene operably linked to a promoter consisting of a basal promoter and binding sites for said DNA binding domain, whereby expression of said reporter gene is altered if said library comprises a polypeptide which modulates activation of said transcription factor activation domain;

(b) determining whether said reporter gene expression is altered in said cell as a result of contact with said polypeptide library; and

(c) if reporter gene expression is altered, identifying a polypeptide which modulates said reporter gene expression.

6. The method of claim 5, wherein step (c) comprises (i) dividing said library into two or more libraries with less complexity; and (ii) repeating steps (a) and (b) until a polypeptide which modulates reporter gene expression is identified.

7. The method of claim 1, 3, or 5, wherein said contacting comprises expressing a library of DNA molecules in a cell, wherein said library of DNA molecules encodes said library of polypeptides.

8. The method of claim 7, wherein said cell is the same cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter.

9. The method of claim 7, wherein said library of DNA molecules is introduced to said cell by transfection.

10. The method of claim 9, wherein the mean number of said DNA molecules introduced by transfection into said cell is at least 25.

11. The method of claim 9, wherein the mean number of said DNA molecules introduced by transfection into said cell is at least 100.

12. The method of claim 9, wherein the mean number of said DNA molecules introduced by transfection into said cell is at least 500.

13. The method of claim 7, wherein said DNA molecules are expressed from a high-efficiency expression system.

14. The method of claim 1, 3, or 5, wherein said polypeptide is produced by the same cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter.

15. The method of claim 1, 3, or 5, wherein said polypeptide is produced by a cell other than the cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter.

16. The method of claim 1, 3, or 5, wherein said polypeptide is selected from the group consisting of an extracellular ligand, a cell surface receptor, and a signal transduction intermediate.

17. A method for identifying a compound which modulates gene expression from a promoter, said method comprising:

(a) contacting a library of compounds with a cell that expresses a recombinant anti-cell death gene and that comprises a reporter gene operably linked to said promoter, whereby expression of said reporter gene is altered if said library comprises a compound which modulates gene expression from said promoter;

(b) determining whether said reporter gene expression in said cell is altered as a result of contact with said compound library; and

(c) if said reporter gene expression is altered, identifying a compound from said library which modulates said reporter gene expression.

18. The method of claim 17, wherein step (c) comprises (i) dividing said library into two or more libraries with less complexity; and (ii) repeating steps (a) and (b) until a compound which modulates gene expression from a promoter is identified.

19. The method of claim 1, 3, 5, or 17, wherein said promoter is a heterologous promoter.

20. The method of claim 1, 3, 5, or 17, wherein said promoter is derived from a mammal and said library of polypeptides comprises polypeptides derived from a bacterium or a virus.

21. The method of claim 1, 3, 5, or 17, wherein said reporter gene is GFP.

22. The method of claim 1, 3, 5, or 17, wherein said anti-cell death gene is selected from the group consisting of bcl family members, IAP family members, and crmA.

23. The method of claim 1, 3, 5, or 17, wherein said cell is selected from the group consisting of CHO, CD-1, Cos, 293, HeLa, BHK, or L cells.

24. A method for determining whether a compound modulates NF-kB biological activity, said method comprising the steps of:

a) providing a cell expressing a BCMA polypeptide;

b) contacting said cell with a candidate compound; and

c) measuring the level of expression of said BCMA polypeptide in said cell, wherein a change in the level of expression of said BCMA polypeptide in said cell, relative to a cell not contacted with said candidate compound, identifies said candidate compound as a compound that modulates NF-kB biological activity.

25. A method for determining whether a compound modulates BCMA biological activity, said method comprising the steps of:

a) providing a BCMA polypeptide;

b) contacting said polypeptide with a candidate compound; and

c) measuring the level of biological activity of said BCMA polypeptide, wherein a change in the level of biological activity of said BCMA polypeptide, relative to a polypeptide not contacted with said candidate compound, identifies said candidate compound as a compound that modulates BCMA biological activity.

26. The method of claim 25, wherein said BCMA polypeptide is in a cell.

27. The method of claim 25, wherein said BCMA polypeptide is in a cell-free system.

28. The method of claim 25, wherein said BCMA biological activity is the modulation of NF-kB biological activity.

29. The method of claim 28, wherein said NF-kB biological activity is the modulation of cell death.

30. The method of claim 25, wherein said BCMA polypeptide comprises a polypeptide sequence having substantial identity to amino acids 98-164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2).

31. A method for determining whether a compound modulates NF-kB activity, said method comprising the steps of:

a) providing a BCMA polypeptide;

b) contacting said polypeptide with a candidate compound; and

c) detecting the binding of said candidate compound to said polypeptide, wherein a candidate compound that binds to said polypeptide is a compound that modulates NF-kB biological activity.

32. The method of claim 31, wherein said BCMA polypeptide comprises a polypeptide sequence having substantial identity to amino acids 98-164 of human BCMA (SEQ ID NO: 1) or amino acids 97-163 of mouse BCMA (SEQ ID NO: 2).

32. A substantially purified polypeptide comprising a polypeptide sequence having substantial identity to amino acids 98-164 of human BCMA (SEQ ID NO: 1) and not having amino acids 1-54 of human BCMA (SEQ ID NO: 1).

33. The polypeptide of claim 32, wherein said polypeptide modulates NF-kB activity.

34. The polypeptide of claim 32, wherein said polypeptide consists of amino acids 98-164 of human BCMA (SEQ ID NO: 1).

35. A substantially purified polypeptide comprising a polypeptide sequence having substantial identity to amino acids 97-163 of mouse BCMA (SEQ ID NO: 2) and not having amino acids 1-49 of mouse BCMA (SEQ ID NO: 2).

36. The polypeptide of claim 35, wherein said polypeptide

modulates NF-kB activity.

37. The polypeptide of claim 35, wherein said polypeptide consists of amino acids 97-163 of mouse BCMA (SEQ ID NO: 2).

38. An NF-kB modulator comprising a polypeptide of claim 32 or 35 covalently linked to a heterologous compound.

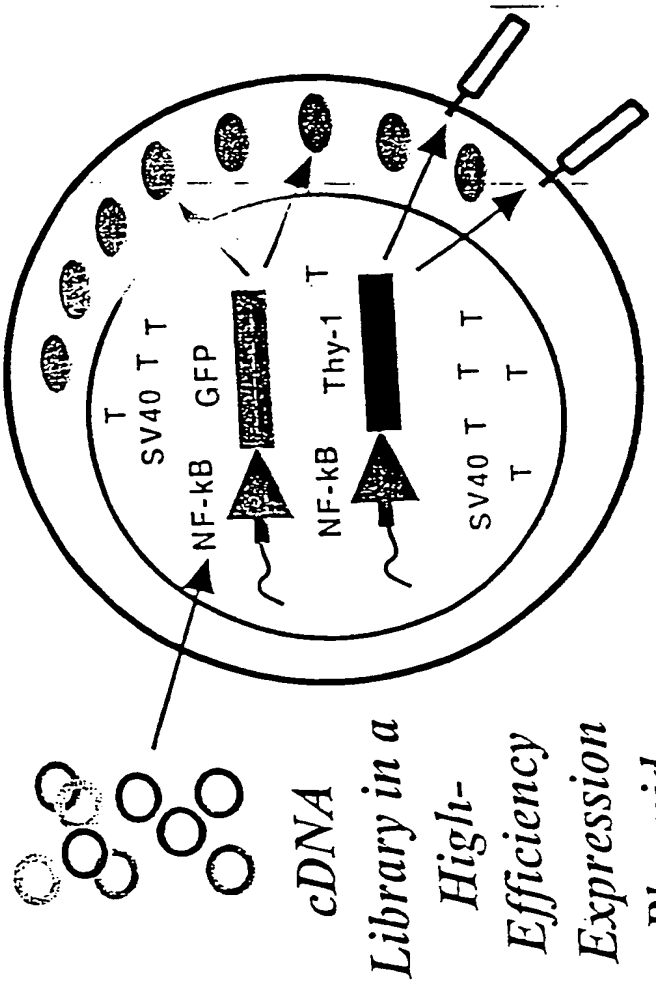
39. The modulator of claim 38, wherein said modulator modulates NF-kB activity.

40. A method for activating NF-kB activity in a cell, comprising contacting said cell with a recombinant BCMA polypeptide having NF-kB activating activity.

41. A method for activating NF-kB activity in a cell, comprising contacting said cell with a recombinant nucleic acid molecule encoding a BCMA polypeptide having NF-kB activating activity.

42. Use of a BCMA polypeptide for preparing a pharmaceutical composition for treating cancer, apoptosis, a viral infection, or an inflammatory response.

43. Use of a BCMA nucleic acid molecule for preparing a pharmaceutical composition for treating cancer, apoptosis, a viral infection, or an inflammatory response.


T
SV40 T T
NF-kB
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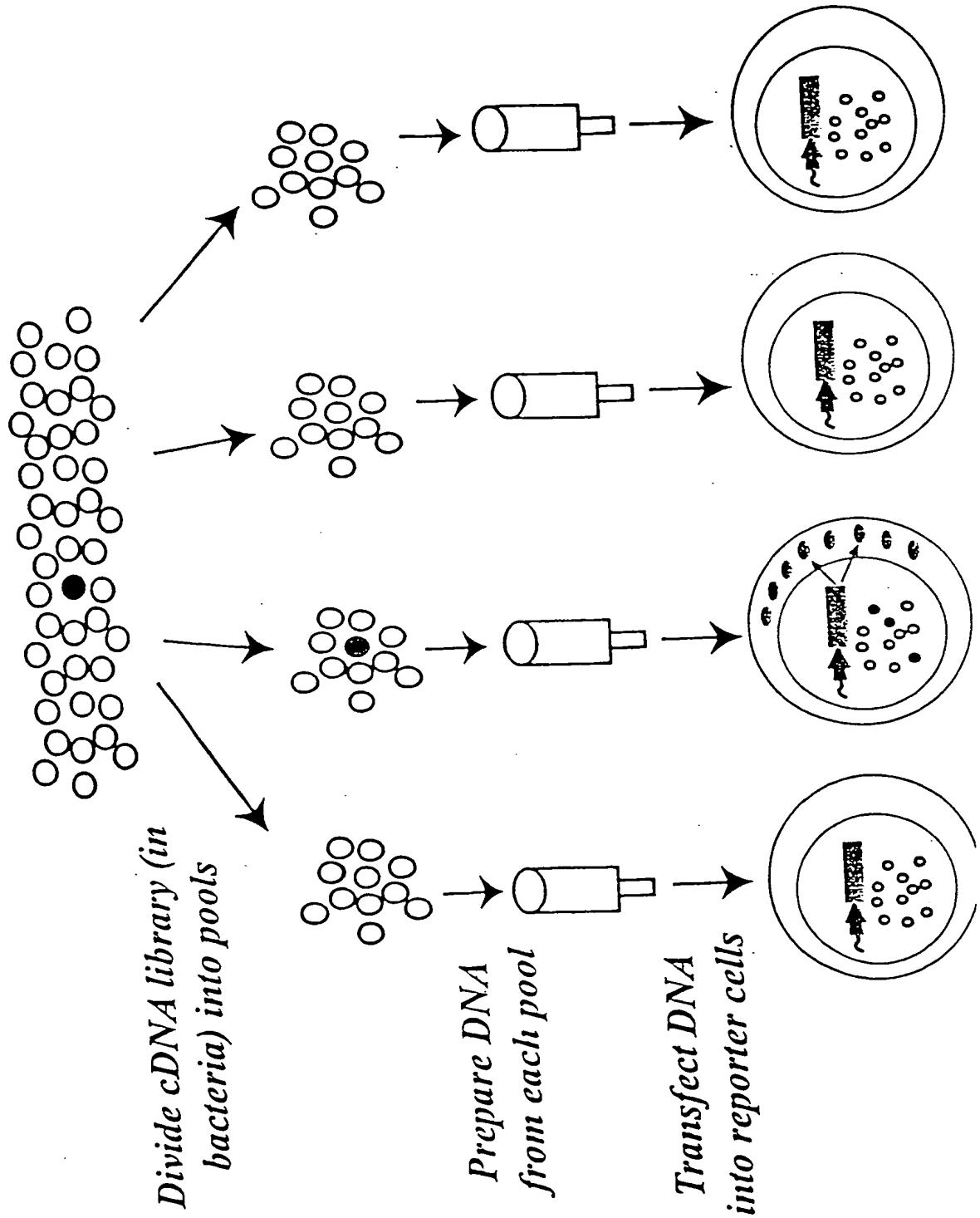
Plasmid

Fig. 1

Fig. 2



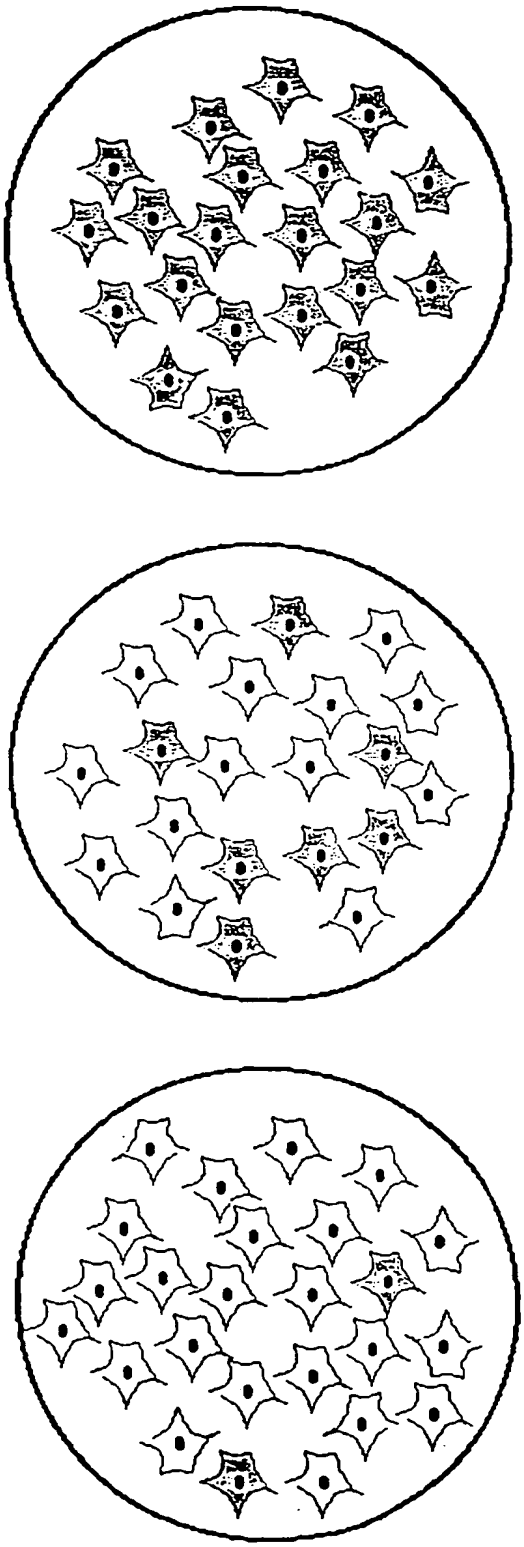


Fig. 3

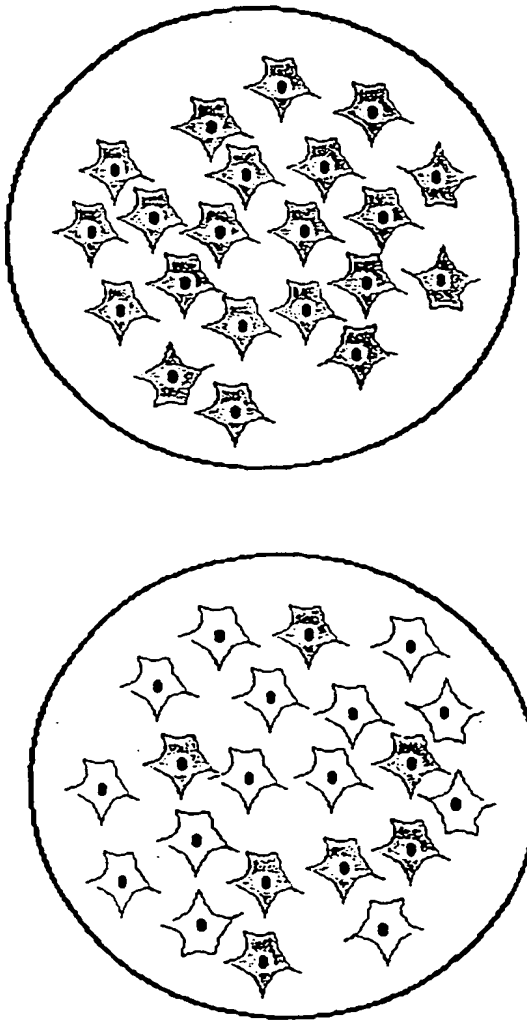


Fig. 4

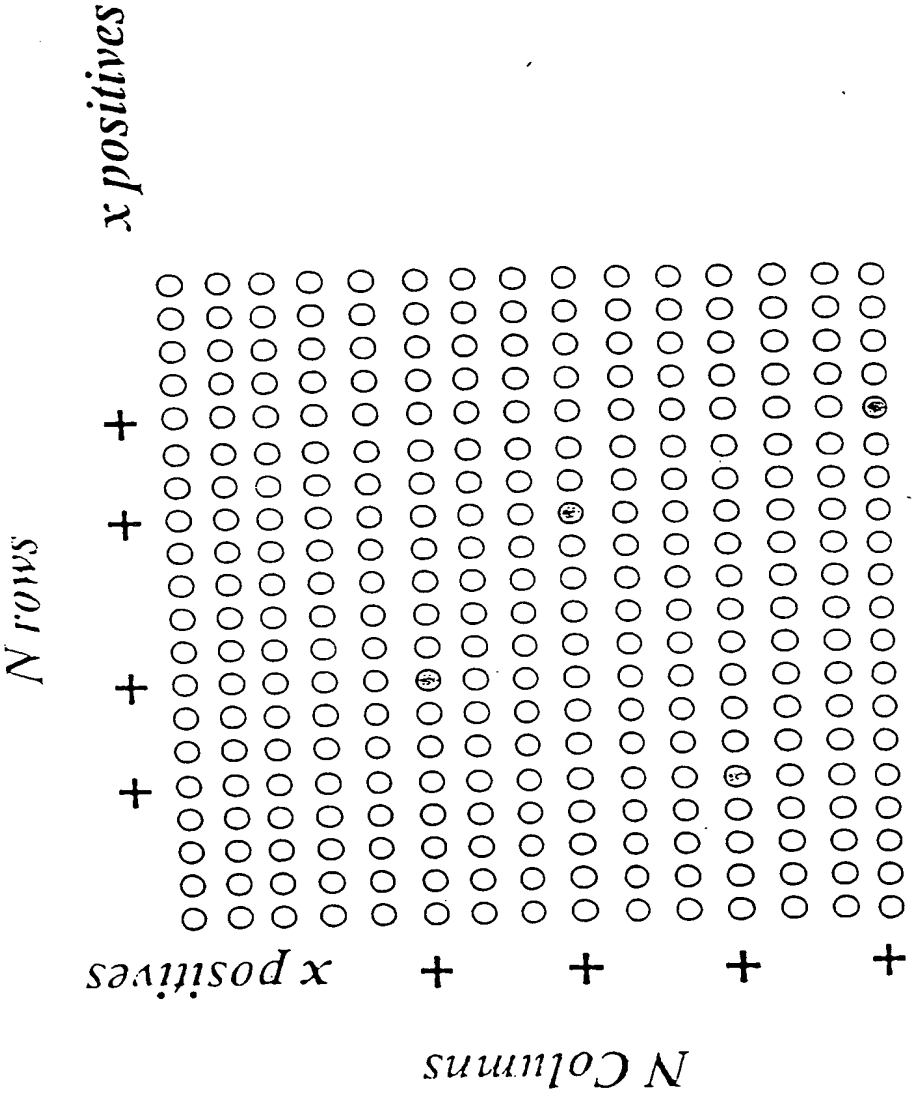
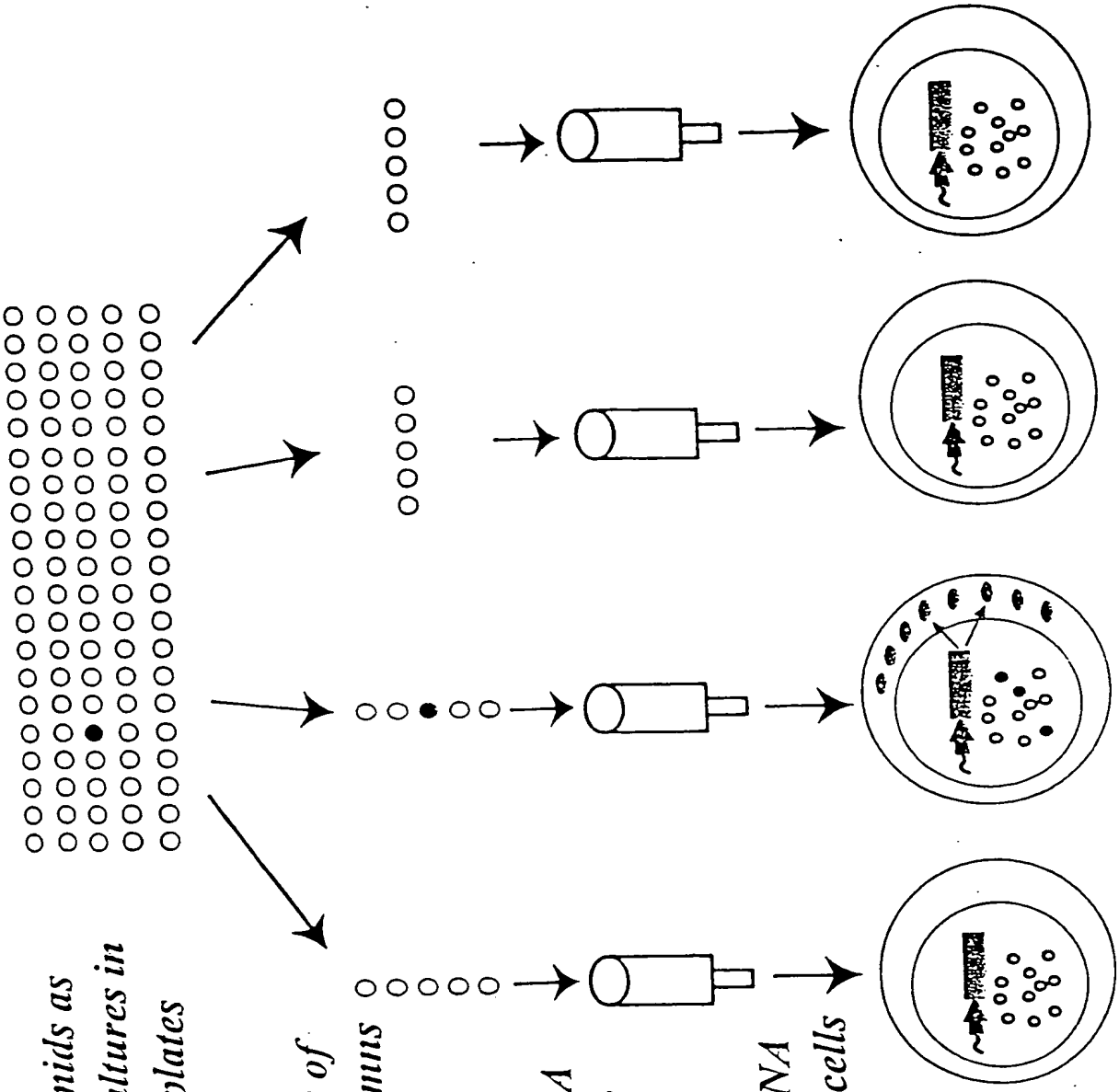


Fig. 5

Fig. 6



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YTVEECTCED CIKSKPKVDS DHCFPLPAME EGATILVTTK TNDYCKSLPA ALSATEIEKS
ISAR (SEQ ID NO: 1)

Fig. 7A
Human BCNA

MAQQCFHSEY FDSLLHACKP CHLRCSNPPA TCQPYCDPSV TSSVKGTYTV LWIFLGLTLV
LSLALFTISF LLRKMNPEAL KDEPQSPGQL DGSAQLDKAD TELTRIRAGD DRIFPRSLEY
TVEECTCEDC VKSKPKGDSD HFFPLPAMEE GATILVTTKT GDYGKSSVPT ALQSVMGMEK
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Fig. 7B
Mouse BCNA

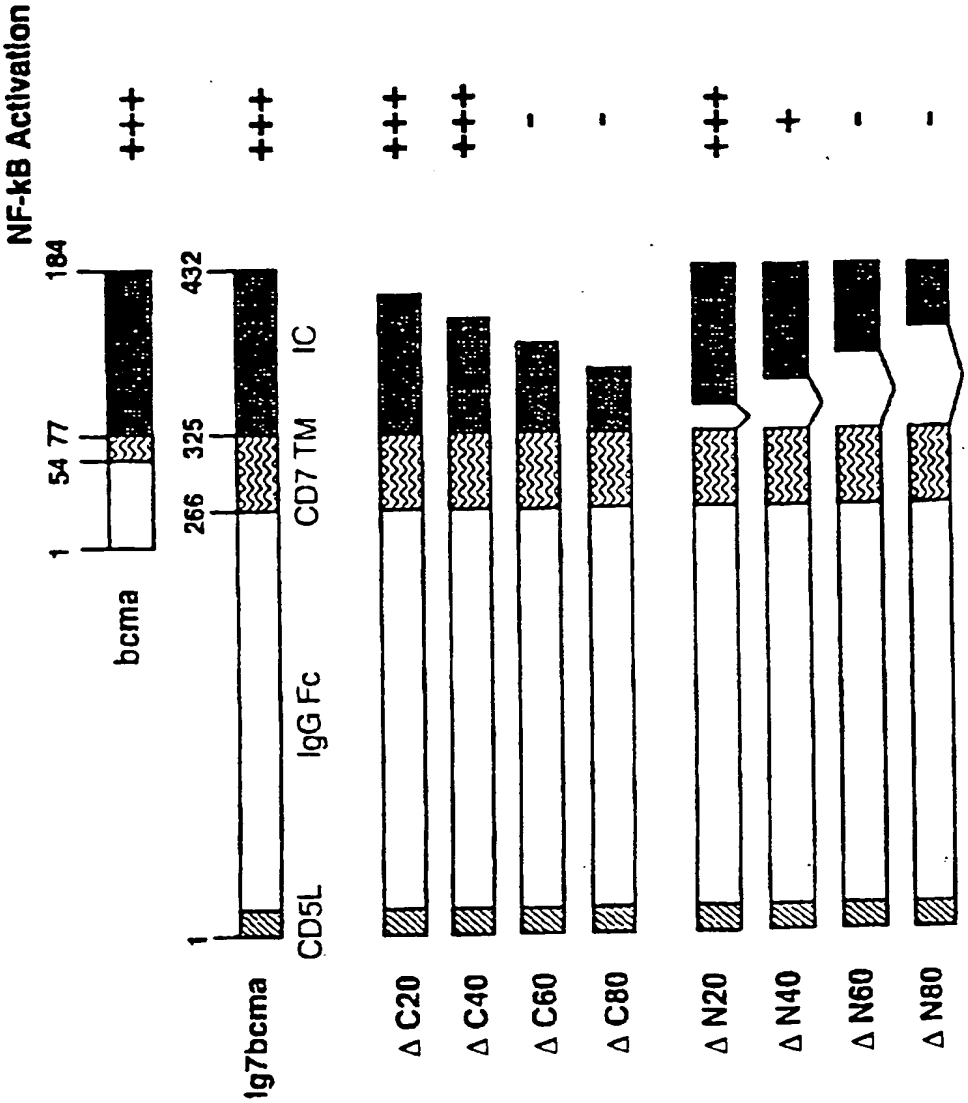
NF-kB Activation
+++
+++
+++
+++
-
-
+++
+
-
-
184
54 77
1
bcma
432
325
266
1
CD5L
IgG Fc
CD7 TM
IC
Ig7bcma
Δ C20
Δ C40
Δ C60
Δ C80
Δ N20
Δ N40
Δ N60
Δ N80

Fig. 8

SEQUENCE LISTING

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20 25 30
Pro Pro Leu Thr Cys Gln Arg Tyr Cys Asn Ala Ser Val Thr Asn Ser
35 40 45
Val Lys Gly Thr Asn Ala Ile Leu Trp Thr Cys Leu Gly Leu Ser Leu
50 55 60
Ile Ile Ser Leu Ala Val Phe Val Leu Met Phe Leu Leu Arg Lys Ile
65 70 75 80
Ser Ser Glu Pro Leu Lys Asp Glu Phe Lys Asn Thr Gly Ser Gly Leu
85 90 95
Leu Gly Met Ala Asn Ile Asp Leu Glu Lys Ser Arg Thr Gly Asp Glu
100 105 110
Ile Ile Leu Pro Arg Gly Leu Glu Tyr Thr Val Glu Glu Cys Thr Cys
115 120 125
Glu Asp Cys Ile Lys Ser Lys Pro Lys Val Asp Ser Asp His Cys Phe
130 135 140
Pro Leu Pro Ala Met Glu Glu Gly Ala Thr Ile Leu Val Thr Thr Lys
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Gln Pro Tyr Cys Asp Pro Ser Val Thr Ser Ser Val Lys Gly Thr Tyr
        35                  40                  45
Thr Val Leu Trp Ile Phe Leu Gly Leu Thr Leu Val Leu Ser Leu Ala
    50                  55                  60
Leu Phe Thr Ile Ser Phe Leu Leu Arg Lys Met Asn Pro Glu Ala Leu
65                  70                  75                  80
Lys Asp Glu Pro Gln Ser Pro Gly Gln Leu Asp Gly Ser Ala Gln Leu
                85                  90                  95
Asp Lys Ala Asp Thr Glu Leu Thr Arg Ile Arg Ala Gly Asp Asp Arg
            100                 105                 110
Ile Phe Pro Arg Ser Leu Glu Tyr Thr Val Glu Glu Cys Thr Cys Glu
        115                 120                 125
Asp Cys Val Lys Ser Lys Pro Lys Gly Asp Ser Asp His Phe Phe Pro
    130                 135                 140
Leu Pro Ala Met Glu Glu Gly Ala Thr Ile Leu Val Thr Thr Lys Thr
145                 150                 155                 160
Gly Asp Tyr Gly Lys Ser Ser Val Pro Thr Ala Leu Gln Ser Val Met
                165                 170                 175
Gly Met Glu Lys Pro Thr His Thr Arg
            180                 185
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04925

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 5, 8, 235.1, 325; 436/518; 530/300; 536/23.1, 23.4, 23.7, 25.32; 935/90, 93, 95, 106

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 1.2, MEDLINE, SCISEARCH, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,874,304 A (ZOLOTUKHIN et al) 23 February 1999(23.02.99), see the entire document.	1-4, 7-16, 19-23
Y	CLEM, R.J et al. Prevention of Apoptosis by a Baculovirus Gene During Infection of Insect Cells. Science. 29 November 1991, Vol. 254, pages 1388-1390, especially page 1388.	1-4, 7-16, 19-23
Y	BIRNBAUM et al. An Apoptosis-inhibiting Gene from a Nuclear Polyhedrosis Virus Encoding a Polypeptide with a Cys/His Sequence Motifs. Journal of Virology. April 1994, Vol. 68, No. 4, pages 2521-2528, especially pages 2523-2524.	1-4, 7-16, 19-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 MAY 2000	Date of mailing of the international search report 19 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer P. PONNALURI Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04925

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PASARELLI et al. A Baculovirus Gene Involved in Late Gene Expression Predicts a Large Polypeptide with a Conserved Motif of RNA Polymerases. Jounal of Virology. July 1994, vol. 68, No. 7, pages 4673-4678, see entire document.	1-4, 7-16, 19-23

Form PCT/ISA/210 (continuation of second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04925

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 7-16, 19-23

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04925

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C12Q 1/00, 1/70, 1/66; C12N 7/00, 5/00, 5/02; G01N 33/554; A61K 38/00; C07H 21/02, 21/04, 21/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/4, 5, 8, 235.1, 325; 436/518; 530/300; 536/23.1, 23.4, 23.7, 25.32; 935/90, 93, 95, 106

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-4, 7-16, 19-23, drawn to a method for identifying a polypeptide which increases gene expression from a promoter.
Group II, claim(s) 5-16, 19-23, drawn to a method of identifying a polypeptide which modulates activation of a transcription factor activation domain.
Group III, claim(s) 17-23, drawn to a method of identifying a compound which modulates gene expression from a promoter.
Group IV, claim(s) 24, drawn to a method of determining whether a compound modulates NF-kB biological activity.
Group V, claim(s) 25-30, drawn to a method of determining a compound which modulates BCMA biological activity.
Group VI, claim(s) 31-32, drawn to a method of determining a compound NF-kB activity.
Group VII, claim(s) 33-37, drawn to a polypeptide which modulates NF-kB activity.
Group VIII, claim(s) 38-39, drawn to an NF-kB modulator.
Group IX, claim(s) 40-41, drawn to a method of activating NF-kB activity in a cell.
Group X, claim(s) 42-43, drawn to use of a BCMA polypeptide or nucleic acid.
The inventions listed as Groups I-X do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of group I is polypeptides which increase gene expression is not present in other groups, and also the polypeptides which increase gene expression from a promoter and method of identifying is known. Thus, the groups lack unity.